Previous work already indicated that group I is involved in determining the structure of the phage coat[1, 3] and that group IV controls the synthesis of the replicating form of the phage DNA.[4] The present work confirms the function of group I and reveals the function of group IIIb.

Summary.—A study of the heat sensitivity of mutants of phage S13 reveals two phage genes that determine the structure of the phage coat. Thus the general function is now understood for three of the five known phage genes.

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*ASSEMBLY OF PHAGE LAMBDA IN VITRO**

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Edgar and Wood[1] have shown recently that it is possible to assemble, *in vitro*, the parts of phage T4 which accumulate in mutant infected cells to form infectious, viable phage particles. This *complementation in vitro* can also be demonstrated for phage lambda, as is shown below.

One would expect lambda to be less complex in structure than T4, since the chromosome of lambda has only one fourth of the DNA content of T4 and presumably contains fewer genes. Indeed, it is found that the physiological functions of the genes of lambda, as revealed by *in vitro* assemblage, divide into two groups only—*head donors and tail donors*, in the terminology of Edgar and Wood.[1] Evidence is presented which suggests that the heads and tails of lambda can unite to form viable phage in the absence of other factors.

Materials and Methods.—Conditional lethal mutants of lambda, similar to the ambers of T4, have been isolated by Campbell,[2] who called them *sus* (suppressor

sensitives). These lambda *sus* mutants grow on the permissive bacteria K12 strain C600, while they do not grow on the restrictive K12 strain 594 (which is strain 3350[2] made resistant to streptomycin[3]). However, these mutants can lysogenize strain 594. After ultraviolet irradiation (UV induction), the bacteria carrying the mutants *A* through *M* lyse, but yield very few infective phage that are revertants or "leakers." The mutants in the genes *N*, *O*, and *P* do not make DNA and do not lyse. The mutant *Q* (maturation-defective[4]) and *R* (no lysozyme[5]) do not lyse after UV induction. The mutants in the genes *N*, *O*, *P*, *Q*, and *R* have not been studied here.

Various strains of 594, each lysogenic for a *sus* mutant defective in one of the genes *A* through *M*, were grown in K medium[6] and were UV-induced when their density was about 2×10^8 bacteria per ml. After induction the cells were maintained at 37°C under strong aeration, and 90 min later chloroform was added. The cultures lysed completely; they were filtered and tested for bacterial sterility. The cultures were kept at 4°C over a period of 2 weeks; only a small decrease in their *in vitro* activity was observed.

These lysates were tested for *in vitro* complementation as described in the text. Active phage were measured by plating on the sensitive, permissive indicator C600.

The following *sus* mutants obtained from Dr. A. Campbell were used: *A*11, *B*1, *C*20, *D*15, *E*4, *G*9, *H*22, *I*2, *J*6, *K*24, *L*63, and *M*87. Each of these mutants complements in simultaneous infection with each other one; the capital letter denotes the gene and the number following it usually denotes the mutant isolate.

The mutant *F*96B, defective in gene *F*, has not been studied in detail because of its high "leakiness."

Results.—Defective lysates of the *sus* mutants *A* through *M* (except *F*) have been tested in all possible pairs for the production of active phage. To 0.8 ml of K medium, 0.1 ml of each of two lysates were added and the mixture was incubated at room temperature for 1 hr. Following appropriate dilutions, the number of active phage produced was measured. The results are given in Table 1. The lysates themselves contain very few phage (although the bacterial density at the time of induction was about 2×10^8 per ml) and the titer does not increase upon incubation. No increase in titer is observed for any combination of the defective mutant lysates *A* through *E*. This is also true of the pairwise combinations *G* through *M*. But all the lysates of the mutants *A* to *E* produce infective phages when mixed with any of the lysates of the mutants *G* to *M*. The titer of the active phage produced in 1 hr, in all the latter cases, shows always more than a hundredfold increase over the pre-existing titer. Nevertheless, even in mixtures of undiluted lysates after a long incubation, the reconstituted phage titer never attains 10^8 per ml. It appears that under the conditions employed, the efficiency of correct assembly is low, for about 10^{10} heads or tails might be expected from the lysis of 2×10^8 cells.

The production of active phages is rapid. When lysates of *sus A* and *sus L* (each previously diluted fivefold in K medium) are mixed in equal volume at room temperature, the phage titer rises within 15 sec by approximately a factor of 100. The reaction initially proceeds linearly with time. Its rate is very insensitive to temperature, since the reactions seem to proceed almost as fast at 4°C as at 30°C.

The phage produced *in vitro* can be tested for their genotype by complementation

TABLE 1

LYSATE COMPLEMENTATION BETWEEN *Sus* MUTANTS DEFECTIVE IN GENES *A* THROUGH *M*

	A	*B*	*C*	*D*	*E*	*G*	*H*	*I*	*J*	*K*	*L*	*M*
A	0.03	0.03	0.06	0.06	0.12	950	40	670	550	1000	1800	550
B	...	0.002	0.03	0.04	0.10	84	16	130	100	280	800	120
C	...	...	0.03	0.07	0.12	180	8	160	110	170	360	50
D	...	...	...	0.04	0.13	260	24	180	210	210	440	50
E	...	...	...	...	0.09	330	9	180	170	120	120	25
G	...	...	...	...	...	0.37	0.39	0.45	0.46	0.68	0.10	0.56
H	...	...	...	...	...	...	0.03	0.96	0.14	0.55	0.70	0.23
I	...	...	...	...	...	...	...	0.07	0.17	0.56	0.75	0.27
J	...	...	...	...	...	...	...	...	0.10	0.52	0.78	0.30
K	...	...	...	...	...	...	...	...	...	0.58	0.60	0.55
L	...	...	...	...	...	...	...	...	...	...	0.68	0.88
M	...	...	...	...	...	...	...	...	...	...	...	0.20

Defective lysates of the various mutants were diluted in the following way: to 0.8 ml of K medium 0.1 ml of each of two lysates was added. The control contained 0.1 ml of a single lysate. The mixtures were incubated for 1 hr at room temperature and the titer of active phage determined. The numbers given in the table are titers divided by 10^9. Lysates prepared on different days varied in their activity and thus the numbers given are indicative of orders of magnitude only.

spot tests. Twenty plaques of the reconstituted phage to be tested were picked each into 1 ml of water. These suspensions were chloroformed and a loopful of each was deposited on an agar plate seeded with the restrictive strain 594. On top of each of these first loopfuls another loopful of the tester phage was placed. These tests showed a clear spot when the two phages complemented each other, while only one or two revertant or leaker plaques appeared in the noncomplementing spots. Of the 35 pairs of mutants yielding reconstituted phage, ten were tested in this fashion. The results were unambiguous: among all the combinations tested, the phage produced *in vitro* were always of the genotype *G* to *M* and never of the genotype *A* to *E*. This finding is also supported by direct inspection of the plaques produced by the reconstituted phage, for a number of the different *sus* mutants form plaques of different morphology.

Dove[4] has shown that the mutants *A* through *M* make large amounts of lambda DNA after induction of the restrictive lysogenic host. However, in the Kaiser[7] assay system, the DNA of *A* through *F* never becomes infectious, while *G* through *M* allow synthesis of infectious lambda DNA. The mutants defective in genes *A* through *F* are blocked in the conversion from newly synthesized lambda DNA to infectious DNA. These genes may thus control the proteins involved in the maturation of DNA (Dove and Weigle[8]). Dove[9] has observed heads of lambda in electron micrographs of *G* to *M* lysates. Our observation that the reconstituted phages have the genotype *G* to *M* suggests that these genes produce competent heads and are defective in tail assembly, and conversely that *A* to *F* are blocked in head morphogenesis but produce competent tails.

Further evidence to support these conclusions comes from attempts to isolate the

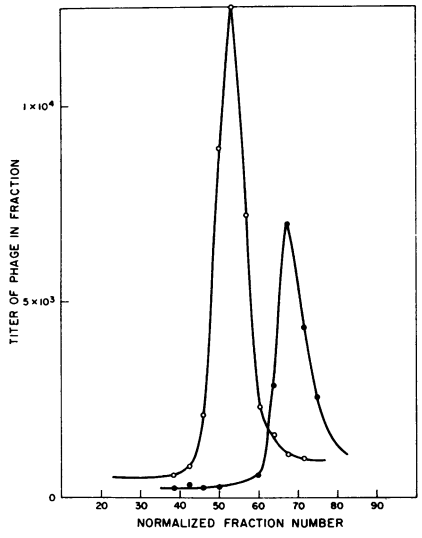

FIG. 1.—Sucrose gradient centrifugation of defective lysate *sus L* with whole phage lambda clear added as a marker. After centrifuging at 20,000 rpm for 22 min, drops were collected from the bottom of the tube. Each fraction was mixed with a defective *sus A* lysate and after 1 hr of incubation at room temperature the titer of active phage produced in each fraction was determined. Filled circles give the titer of the lambda marker.

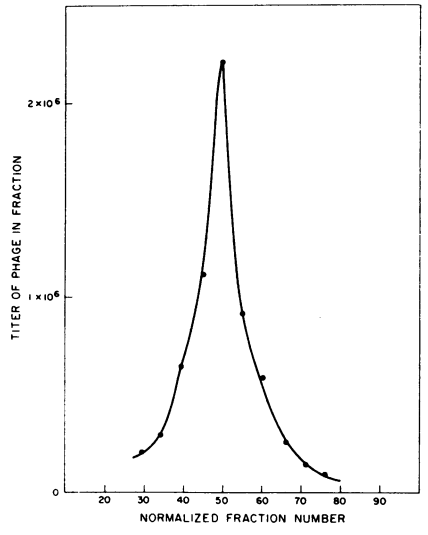

FIG. 2.—Sucrose gradient centrifugation of defective *sus A* lysate. After centrifuging at 37,000 rpm for 80 min, the fractions were collected from the bottom of the tube. Each fraction was mixed with a defective *sus L* lysate and after 1 hr of incubation at room temperature, the titer of active phage was determined.

active fractions of the various lysates by sucrose density gradient velocity sedimentation.

In the procedure we employed, 0.25 ml of a lysate containing presumably heads was placed on top of a 5-ml sucrose gradient (20%–5% in 0.07 *M* phosphate buffer). The centrifugation in a Spinco swinging bucket (SW39) lasted 22 min at 20,000 rpm. The bottom of the tube was pierced and the emerging drops were collected (2 drops per tube) in 0.2 ml of a presumed tail containing lysate like *sus A*. After 1 hr of incubation at room temperature the titer of the phage in each tube was measured. The results are shown in Figure 1. The head containing lysate (*sus L*) had been mixed with a small amount of λ clear as a marker. The activity of the presumed heads sediments faster than whole phage (which are slowed down by their tails).

The result of the inverse experiment, sedimenting *tails* (*sus A*) and collecting fractions in a lysate which contributes heads (*sus L*), is shown in Figure 2. In this case much more intensive centrifugation was used lasting 80 min at 37,000 rpm. Under these conditions complete phage or heads would be sedimented completely. Our results indicate that the heads have a sedimentation coefficient of $S_{20,w} = 650$, while for tails the value is $S_{20,w} = 47$, and for whole lambda phage it is $S_{20,w} = 410$.

The comparison of the results of Figures 1 and 2 shows that the titer of the reconstituted phage is much higher when tails are centrifuged and collected into head lysates than the reverse. This is probably due to instability of the heads.

By sedimenting out the phage and the heads, one can determine whether a leaky defective lysate from *sus F* contains *tail* activity or if a wild-type lysate contains an excess of *tails*. It is found that both lysates do contain a large amount of *tail* activity. The tail activities of the defective lysates *A* through *F* and of the wild-type lysate have the same $S_{20,w}$.

To determine whether the union of heads and tails of lambda requires additional factors present in the lysates, a head-donating lysate (*sus L*) and a tail-donating lysate (*sus A*) were sedimented separately in sucrose. The times and velocities of centrifugation were chosen so that the activities in both gradients would occur in the same fractions. Each head fraction was mixed with the corresponding tail fraction. A peak of activity was found where the two activities overlap. This activity was comparable to that found when the peak head fraction was tested against the unpurified tail lysate. This result suggests that all factors necessary for active phage formation sediment with the heads and the tails, respectively.

Discussion and Summary.—The results of *in vitro* complementation tests involving lysates of mutants of lambda defective in 12 of the 13 genes concerned with virus morphogenesis reveal but two complementing types. Lysates of the mutants *A* through *E* complement with lysates of mutants *G* through *M* to form active phage particles. The mutants *G* through *M* contribute the genome already contained in head membranes. The mutants *A* through *E* presumably contribute the tail. In the terminology of Edgar and Wood, mutants *A* through *E* are *tail donors* and *G* through *M* *head donors*. These conclusions are supported by sedimentation experiments in sucrose gradients showing that the *activity* in the lysates *A* through *E* sediments with a velocity expected for tails, while the *activity* in *G* through *M* lysates sediments with a velocity expected for heads without tails.

Genes *A* through *E* seem to be concerned with head formation so that defective lysates contain tails but no heads, while genes *G* through *M* are concerned with tail formation so that defective lysates contain heads but no tails. It is probable that *in vitro* the heads and tails can unite spontaneously to form active phage particles.

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